

Diagnosing Covid-19 by CRISPR Technology

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ABSTRACT

In December 2019, a series of patients with symptoms resembling viral pneumonia with unknown etiology detected in Wuhan, China. A novel coronavirus is identified by the disease symptoms which resembles two families of coronavirus and known as COVID-19. COVID-19 is a disease which is associated with the infection of SARS-CoV-2. It becomes a pandemic disease globally because it spreads. CRISPR is a technology can be used for the diagnosis, which is a rapid, easy to implement and an accurate technology. CRISPR -Cas protein-based assay is used for the detection of SARS-CoV-2 infection. CRISPR based assay is an alternative method for other diagnostic techniques like RT-PCR assay because it is faster and more accurate than other techniques.

KEYWORDS: COVID-19, SARS-CoV-2, CRISPR, RT-PCR, nCoV, ICTV, MERS-CoV, ACE2, Cas Protein, Cas9, IGIB

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INTRODUCTION

Beginning in December 2019, a series of patients with symptoms resembling viral pneumonia with unknown etiology detected in Wuhan, China. China has first reported to the WHO country office in China on 31 December 2019. However, the cause of disease with a clinical manifestation of infected patients include fever (77-98%), cough (59-81%), fatigue (38-98%) Malaga (11-34%), sputum production (28-56%), and headache (6-33%) and it spread rapidly throughout China. In the month of January 8, 2020, eventually novel coronavirus (nCoV) was identified as the seventh member of the Coronaviridae by the Chinese Centers for Disease Control and Prevention and disease symptoms resemble the other two families of coronavirus. A study group of the international committee of Taxonomy of Viruses (ICTV) proposed the name as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting disease termed 2019 novel coronavirus (2019-nCoV). On 30 January 2020 WHO declared it as an international public health emergency and 11 February 2020, WHO announced the name of the new coronavirus disease COVID-19.

In November 2002, the first cases of the coronavirus family reported as severe acute respiratory syndrome coronavirus (SARS-CoV) emerge in Guangdong, China that infected 8000 people and 774 deaths. Second reported, in 2012, known as Middle East respiratory syndrome coronavirus (MERS-CoV) which was first detected in Saudi Arabia. However, these pathogenic viral strains isolated from camels have affected 2494 individuals and caused 858 mortalities.

According to an epidemiological report severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2 share many similar clinical symptoms. The most

severe type of coronavirus can affect the respiratory tract in immunocompetent hosts and can cause viral pneumonia. It may also affect the gastrointestinal system, body organs such as heart, kidney, liver and central nervous system leading to multi-organ failure. However, patients infected with SARS and MERS have gastrointestinal symptoms (one third) than COVID19 patients. A recent study reported that male reproductive systems are susceptible to infection and dramatic changes in sex hormones have been Observed in patients suggesting gonadal function impairment. Different phases of the epidemic condition have also influenced the clinical characteristics of COVID-19.

The epidemiologic study by another group has reported the surface stability of SARS-CoV-2 and SARS CoV-1 under the experimental circumstances and both are similar. Similarly, an indication was made that the epidemiologic characteristic feature of these viruses probably arises from other factors. Thus, the result of the virus can remain viable and contagious in aerosols for several hours and days on surfaces (depending on the inoculum shed). As seen that more pathogenic viruses that are well transmitted between humans can usually be contained effectively implicated through syndromic (high fever) surveillance and contact tracing, for example, SARS-CoV-2 and, more recently, Ebola virus.

1. Evolution of 2019-nCoV:

Coronavirus is a class of genetically diverse viruses found in a wide range of host species, including birds and mammals. Coronaviruses are members of the subfamily Coronavirinae in the family *Coronaviridae* and the order Nidovirales. Based on their phylogenetic relationship, this subfamily consists of four genera Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. The

alphacoronaviruses and betacoronaviruses cause infection in gastroenteritis of human. The gamma coronaviruses and delta coronaviruses infect birds, but some of them can also be responsible to infect mammals. However, based on current sequence databases, domestic animals may have important roles as intermediate hosts that enable virus transmission from natural hosts to humans. Also, domestic animals themselves can suffer a disease caused by bat-borne or closely related coronaviruses. A study reported that 4 out of 9 betacoronavirus species were identified only in bats.

Structure and pathogenic mechanism:

COVID-19 is a spherical or pleomorphic enveloped particle containing single-stranded RNA positive-sense RNA viral genome, encodes several structural proteins, including the spike and glycosylate protein (S). This S protein mediates host cell invasion by SARS-CoV-2 via binding to a receptor protein called angiotensin-converting enzyme 2 (ACE2) located on the surface membrane of host cells inducing host immune responses. To complete the entry of into the host cell Following initial process, the interaction between viral S protein use the cellular attachment factor ACE2 and the cellular protease TMPRSS2 for their activation is of significant interest since it initiates the infection process.

There are many other viral proteins in the membrane and the most abundant structural protein is the membrane glycoprotein. The 3D structure of viral S protein determines by Cryogenic Electron Microscopy has revealed that the binding affinity of SARS-CoV-2 S protein to ACE2 is about 10–20 times higher than that of SARS-CoV S protein. It is speculated that this may contribute to the reported higher transmissibility and contagiousness of SARS-CoV-2 as compared to SARS-CoV.

Researchers identified that the COVID19 geometrical similarity differs from SARS-CoV about 79% and MERS-CoV about 50%. Sequence alignment and homology modelling show that SARS-CoV and SARS-CoV-2 share a highly conserved receptor-binding domain (RBD). Current evidence reported that S protein of SARS-CoV-2 functionally divides into two subunits which consist of 1253aa and these amino acid shares 75% of identity. These two subunits S1 and S2 play an important role in binding to the receptor and cell membrane fusion activity, respectively. S1 is further divided into two domains: first, N-terminal domain (NTD) and the C-terminal domain (CTD). Each domain has a different function like CTD functions as the RBD and is responsible for binding ACE2 and entering cells. Whereas the NTD display a structural fold as human galectins, galactose-binding lectins, and hence, in other cases, a carbohydrate present at the cell surface assist as an attachment factor. After binding to ACE2 receptor, viral S protein catalyses fusion of the viral and cellular membrane to allow access of the viral genome to the cytosol, starts replication and infect host.

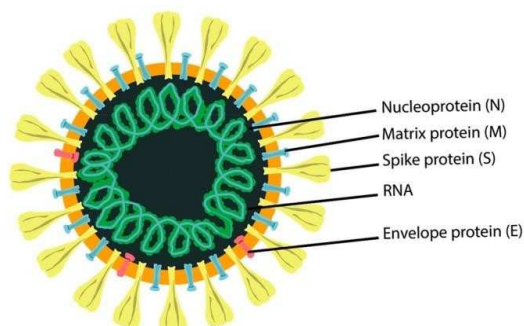


Fig Structure of COVID -19

2. CRISPR:

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a technique of gene editing in which the Cas9 protein and several other Cas proteins are utilized along with the specific guide RNAs and used to either disrupt host genes or insert sequences of interest. Initially this technique is used in bacteria as an adaptive immunity response, then CRISPR have been utilized in the various biological fields as a new alternative to genome engineering. As compared to other diagnosis technique, CRISPR is a cheaper alternative to other gene editing techniques such as zinc fingers and is quickly being adopted as the best technique for gene editing and diagnosis.

About Cas:

Cas (CRISPR associated protein) is a naturally occurring enzyme found in some bacteria that is used for immunity. Cas protein works by using guide RNA with short sequences complementary to potential foreign DNA, combating infection. This mechanism has similarities to RNA interference found in many eukaryotes. Because of its capabilities, Cas9 has been used recently in experiments to serve as a genome editing tool.

How CRISPR works:

1. First, the Cas protein with designing guide RNA [s] are injected into developing embryos.
2. Once they get inside the cell body, Cas protein searches for the targeted DNA sequence that is complementary to the sequence in the designed guide RNA.
3. The DNA helix is unwinding by Cas protein when the sequence is recognized.
4. After the DNA has been unwound, Cas9 creates a double stranded breaks in the cell's DNA.
5. After that, the double-stranded break is repaired by the repair enzyme along with the integration of new experimental DNA.

3. CRISPR as a Diagnosis Technique for COVID-19:

There is various diagnosis method for COVID-19 based on the symptoms, the techniques are chest CT (Computed tomography scan) scans and QRT-PCR (Quantitative reverse transcription polymerase chain reaction). Currently, the QRT-PCR technique is the standard molecular detection method for COVID-19 (SARS-CoV-2), approved by the CDC and World Health Organization (WHO). Recently, a coronavirus rapid detection method is proposed by the researchers which is based on CRISPR/Cas system with the advantages of sensitivity, specificity, rapidity, and simplicity.

CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats) is an adaptive immune system in archaea and bacteria against foreign genetic elements such as phages. Recently, CRISPR/Cas is used as a powerful gene editing tool and treatment for genetic diseases and cancers. In this technique, we used a protein linked with the guide RNA, which helps it attaches to the target site by a guide RNA and cleaved of the target sequence. There are several types of Cas proteins that have different properties. Among them, Cas9 protein has received more attention for gene editing whereas, Cas12a and Cas13a have been more efficient in diagnosis of diseases. Cas12a is DNA-specific, but Cas13a works with RNA, which makes it convenient in the detection of SARS-CoV-2.

Diagnosis

Recently, Zhang *et al* reported specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) technology,

which is a CRISPR/Cas13 based nucleic acid detection technique for rapid detection of SARS-CoV-2.

In this diagnostic technique, the ORF 1ab and S protein genes in the genome of coronavirus are targeted. This SHERLOCK technique uses ssRNA or ssDNA reporter used is labelled with a quenched green fluorescent molecule.

- First, the RNA is purified from the patient sample as in the other diagnostic techniques.
- Cas 12a/ Cas 13 proteins identifies the targeted sequence that is ORF 1ab and S protein genes in the genome of corona virus and binds to it.
- An ssDNA reporter labelled with a quenched green fluorescent molecule was introduced, which will be cleaved by Cas protein when there is a nucleic acid of

SARS-CoV-2 in the detection system.

- When the fluorescent molecule is cleaved, this results in the emission of green fluorescence can be seen with the naked eye under 485 nm light.
- The viral genome is detected at attomolar concentration using this technology.

To improve the sensitivity of the reaction, targeted DNA or RNA can be amplified by Recombinase Polymerase Amplification (RPA) or reverse transcriptase-RPA (RTPA). Application of CRISPR/Cas13 based diagnosis or SHERLOCK for SARS-CoV-2 detection is much faster than detection by QRT-PCR and has high sensitivity. So, it can replace QRT-PCR technique.

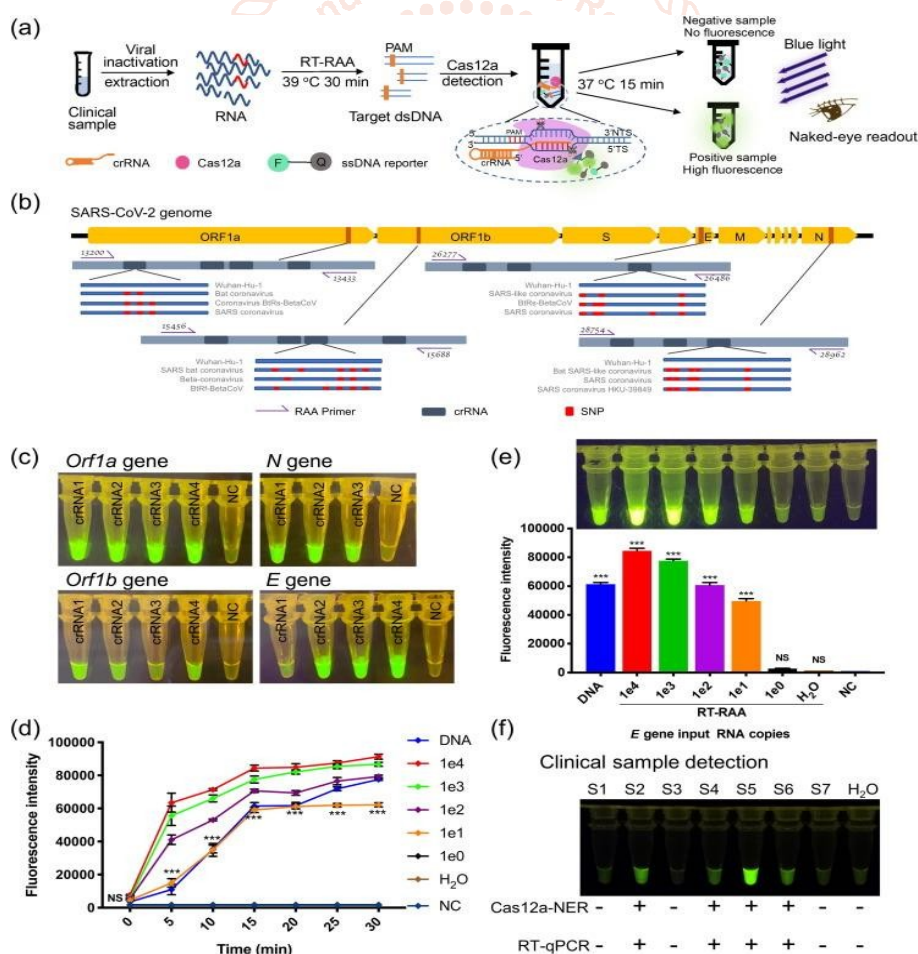
4. PROCEDURE:

RNA is isolated and purified from the patient sample.

After purification of RNA, RT-RAA is performed which is reverse-transcription recombinase- aided amplification assay. It takes approx. 30 min at 39 degree Celsius.

Add ssDNA reporter labelled with fluorescent to the amplified sample and incubate for 15 min. At 37 degree Celsius.

Then observe the sample, if there is emission of green fluorescence then it will be a positive sample and if there is no fluorescence then it will be a negative sample.



CRISPR/Cas12a-based detection with naked eye readout (CRISPR/Cas12a-NER) of SARS-CoV-2. (a) Schematic of Cas12a-based assay for rapid and visual nucleic acid detection. After 30 min of RT-RAA reaction, the Cas12a enzyme targets cleavage for an additional 15 min. The green fluorescent signals can be observed by the naked eye under blue light with a wavelength of 485 nm. (b) Four target gene fragments and SARS-CoV-2-specific crRNAs location on the genome. (c) Validation of crRNA specifically reacts with the target gene. (d) Time course of E gene detection with E-crRNAmix on gradient diluted input RNAs from 1e4 to 1e0 copies. (e) Sensitivity assay using E-crRNAmix to detect gradient diluted E gene RNA from 1e4 to 1e0 copies. (f) Diagnostic accuracy of CRISPR/Cas12a-NER on the diagnosis of COVID-19 clinical samples. ***: statistical significance; NS: not significance.

5. INDIA'S CRISPR BASED COVID -19 TEST- 'FELUDA'

On 19 SEP 2020, the Tata CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) test, powered by CSIR-IGIB (*Institute of Genomics and Integrative Biology*) FELUDA, received regulatory approvals today from the Drug Controller General of India (DCGI) for commercial launch, as per ICMR guidelines, meeting high quality benchmarks with 96% sensitivity and 98% specificity for detecting the novel coronavirus.

This test is developed which uses cutting-edge CRISPR technology for detection of the genomic sequence of SARS-CoV-2 virus.

The Tata CRISPR test is the world's first diagnostic test to deploy a specially adapted Cas9 protein to successfully detect the virus causing Covid-19. This marks a significant achievement for the Indian scientific community, moving from R&D to a high-accuracy, scalable and reliable test in less than 100 days. The Tata CRISPR test achieves accuracy levels of traditional RT-PCR tests, with quicker turnaround time, less expensive equipment, and better ease of use. Moreover, CRISPR is a futuristic technology that can also be configured for detection of multiple other pathogens in the future. The effort is the result of a fruitful collaboration between the scientific community and industry. The Tata Group has worked closely with CSIR-IGIB and ICMR to create a high- quality test that will help the nation ramp up Covid-19 testing quickly and economically, with a 'Made in India' product that is safe, reliable, affordable, and accessible. Scientists from Delhi based - Institute of Genomics and Integrative Biology (IGIB) would work together with Tata Sons to develop India's first paper-strip test for rapid diagnosis of Covid-19.

RESULT AND CONCLUSION:

COVID-19 is a pandemic disease, and in this report we introduce a rapid detection method of coronavirus which is CRISPR-based technology. This assay can detect the viral gene in 45 min. and does not need any special instrument and professional operations. Also, the result of CRISPR assay can be observed by naked eye because a fluorescent light is emitted in the positive sample. CRISPR based technology is more advantageous than qPCR and other detection techniques in sensitivity, specificity, rapidity, and simplicity. So, CRISPR based assay provides a simple and reliable on-site detection method.

REFERENCES:

- [1] Agrebi S, Larbi A. Use of artificial intelligence in infectious diseases. *Artificial Intelligence in Precision Health*. 2020; 415-438. DOI: 10. 1016/B978-0-12-817133-2. 00018
- [2] Battegay M, Kuehl R, Tschudin-Sutter S, Hirsch HH, Widmer AF, Neher RA. 2019-novel Coronavirus (2019-nCoV): estimating the case fatality rate-a word of caution. *Swiss Med Wkly* 2020; 150:w20203. [PubMed] [Google Scholar]
- [3] Broughton JP, Deng W, Fasching CL, Singh J, Chiu CY, Chen JS. A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR.
- [4] Chen J. S., Ma E., Harrington L. B. Crispr-cas12a target binding unleashes indiscriminate single-stranded
- dnase activity. *Science*. 2018; 360:436-439. [PMC free article] [PubMed] [Google Scholar]
- [5] Chu D. K. W., Pan Y., Cheng S. M. S. Molecular diagnosis of a novel coronavirus (2019-ncov) causing an outbreak of pneumonia. *Clin Chem*. 2020; 66:549-555. [PMC free article] [PubMed] [Google Scholar]
- [6] Control CfD, Prevention. Frequently Asked Questions and Answers: Coronavirus Disease 2019 (COVID-19) and Pregnancy. Accessed; 2020.
- [7] Creutzburg S. C. A., Wu W. Y., Mohanraju P. Good guide, bad guide: spacer sequence- dependent cleavage efficiency of cas12a. *Nucleic Acids Res*. 2020; 48:3228-3243. [PMC free article] [PubMed] [Google Scholar]
- [8] Cui, J., Li, F. & Shi, Z. Origin, and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 17, 181-192 (2019). <https://doi.org/10.1038/s41579-018-0118-9>
- [9] Gootenberg J. S., Abudayyeh O. O., Lee J. W. Nucleic acid detection with crispr- cas13a/c2c2. *Science*. 2017; 356:438-442. [PMC free article] [PubMed] [Google Scholar]
- [10] Gorbalenya AE. Severe acute respiratory syndrome-related coronavirus-The species and its viruses, a statement of the Coronavirus Study Group. *BioRxiv* 2020.
- [11] Hryhorowicz M, Lipiński D, Zeyland J, Słomski R. CRISPR/Cas9 immune system as a tool for genome engineering. *Arch Immunol Ther Exp (Warsz)* 2017; 65(3):233-240. [PMC free article] [PubMed] [Google Scholar]
- [12] <https://global.infervision.com/>
- [13] <https://robotics.sciencemag.org/content/5/40/eabb5589>
- [14] <https://www.futurithmic.com/2020/03/27/how-ai-might-help-diagnose-track-covid-19/>
- [15] https://www.nejm.org/doi/full/10.1056/NEJMc2004973?query=recirc_mostViewed_railB_article
- [16] <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>
- [17] hYang Z, Zeng Z, Wang K, et al. Modified SEIR and AI prediction of the epidemics trend of COVID-19 in China under public health interventions. *J Thorac Dis*. 2020; 12(3):165- 174. doi:10. 21037/jtd. 2020. 02. 64
- [18] Jin Y. H., Cai L., Cheng Z. S. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-ncov) infected pneumonia (standard version) *Mil Med Res*. 2020; 7:4. [PMC free article] [PubMed] [Google Scholar]
- [19] Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res* 2020; 7(1):4. [PMC free article] [PubMed] [Google Scholar]
- [20] Jiumeng Sun., Wan-Ting He., et al., COVID-19:

- Epidemiology, Evolution, and Cross- Disciplinary Perspectives, Trends in Molecular Medicine, 2020,
- [21] Juan A. Siordia, Epidemiology and clinical features of COVID-19: A review of current literature, Journal of Clinical Virology, Volume 127, 2020, 104357, ISSN 1386-6532,
- [22] Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. Author Correction: SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc 2020; 15(3):1311. [PubMed] [Google Scholar]
- [23] Kim H. K., Song M., Lee J. *In vivo* high-throughput profiling of crispr-cpf1 activity. Nat Methods. 2017; 14:153–159. [PubMed] [Google Scholar]
- [24] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrobial Agents 2020; 55(3):105924. [PMC free article] [PubMed] [Google Scholar]
- [25] Li SY, Cheng QX, Wang JM, Li XY, Zhang ZL, Gao S, et al. CRISPR-Cas12a-assisted nucleic acid detection. Cell Discov 2018; 4(1):20. [PMC free article] [PubMed] [Google Scholar]
- [26] Li Y, Glass Z, Huang M, Chen ZY, Xu Q. Ex vivo cell based CRISPR/Cas9 genome editing for therapeutic applications. Biomaterials 2020; 234:119711. [PMC free article] [PubMed] [Google Scholar]
- [27] Lu R., Zhao X., Li J. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020; 395:565–574. [PMC free article] [PubMed] [Google Scholar]
- [28] Metsky HC, Freije CA, Kosoko-Thoroddsen T-SF, Sabeti PC, Myhrvold C. CRISPR- based COVID-19 surveillance using a genomically-comprehensive machine learning approach. BioRxiv 2020.
- [29] Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, et al. Field-deployable viral diagnostics using CRISPR- Cas13.
- Science 2018; 360 (6387):444–448. [PMC free article] [PubMed] [Google Scholar]
- [30] Rasmussen SA, Smulian JC, Lednický JA, Wen TS, Jamieson DJ. Coronavirus Disease 2019 (COVID-19) and Pregnancy: What obstetricians need to know. Am J Obstet Gynecol 2020. [Epub ahead of print]. [PMC free article] [PubMed]
- [31] Sexton NR, Smith EC, Blanc H, Vignuzzi M, Peersen OB, Denison MR. Homology-based identification of a mutation in the coronavirus RNA-dependent RNA polymerase that confers resistance to multiple mutagens. J Virol 2016; 90(16):7415–7428. [PMC free article] [PubMed] [Google Scholar]
- [32] Shan F, Gao Y, Wang J, Shi W, Shi N, Han M, et al. Lung Infection Quantification of COVID-19 in CT Images with Deep Learning. arXiv preprint arXiv: 200304655. 2020.
- [33] Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323(11):1061–1069. [PMC free article] [PubMed] [Google Scholar]
- [34] World Health Organization Coronavirus disease 2019 (COVID-19): situation report, 45. 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200305-sitrep-45-covid-19.pdf?sfvrsn=ed2ba78b_4
- [35] Wu F., Zhao S., Yu B. A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579:265–269. [PMC free article] [PubMed] [Google Scholar]
- [36] Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. JAMA 2020; 323(13):1239–1242. [PubMed] [Google Scholar]
- [37] Zhang F, Abudayyeh OO, Jonathan SG. A protocol for detection of COVID-19 using CRISPR diagnostics. [https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20\(updated\).pdf](https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf).